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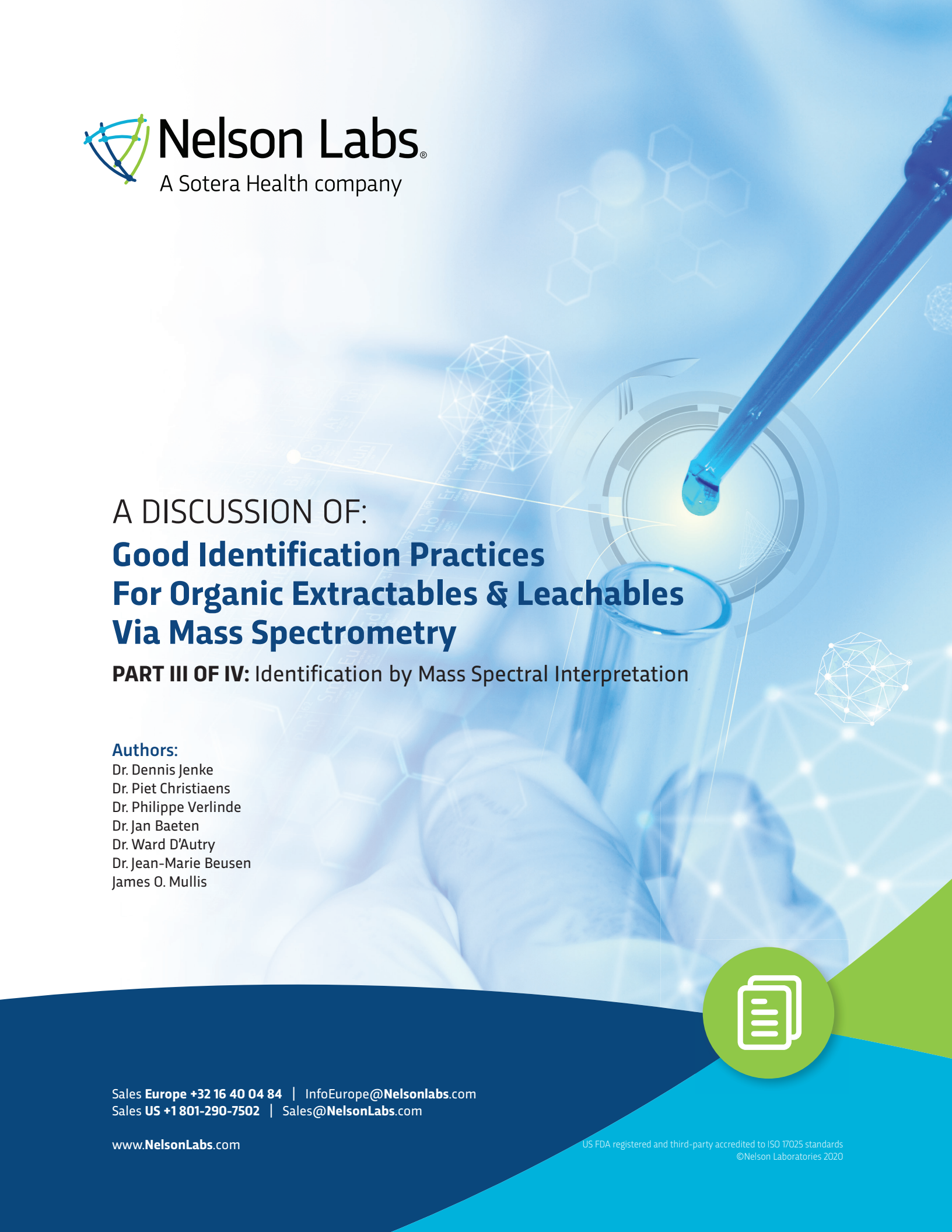

A DISCUSSION OF:

Good Identification Practices For Organic Extractables & Leachables Via Mass Spectrometry

PART III OF IV: Identification by Mass Spectral Interpretation

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PART 3: IDENTIFICATION BY MASS SPECTRAL INTERPRETATION

OPENING THOUGHTS

Identification of extractables and leachables is a critical aspect of reporting these substances for toxicological safety risk assessments, as it is these substances' identity that establishes their inherent toxicity. Nelson Labs has generated a series of white papers focused on the aspect of identification of extractable and leachable compounds surfaced by chromatographic screening analyses. More specifically, a process is proposed by which mass spectral data and other supporting evidence is used to secure, judge, and justify complete and correct identities for all relevant organic extractables or leachables. Part I of this series introduced the concept of identification and established its critical role in safety assessment. In general, Part I described the various means of securing identities, discussed the concept of identification classes and proposed an identification classification, emphasized the importance of confidence in identification, and delineated the identification process via an identification decision tree (*see Figure 6 in Part I of this series on Good Identification Practices: Identification Classes, Process and Practices).* In Part II of the series, the process of securing a compound's identity via mass spectral matching to Mass Spectral libraries was considered; specifically addressing the strengths, points of attention, and potential pitfalls of such an identification strategy.

In this document (Part III), the identification strategy *Mass Spectral Interpretation* is explored. *Mass Spectral Interpretation* is the process of securing a compound's identity solely by expert interpretation of the information that is made available through the compound's mass spectrum. This is an identification strategy that is often applied to the mass spectral information generated from an LC/MS experiment, as there are no universal commercial libraries available that can provide identities based solely on mass spectral matching. However, it can also be necessary to follow this type of approach for GC/MS when mass spectral matching does not lead to a reliable identity for the detected compound.

INTRODUCTION TO MASS SPECTRAL INTERPRETATION

Although tentatively identifying a compound of interest via GC/MS can often be accomplished by mass spectral matching *(or MSM)*, unequivocal identification based solely on *MSM* is not possible. Clearly, there will be situations where a compound's mass spectrum cannot be effectively matched to a library spectrum, meaning that *MSM* fails to provide even a tentative identity for the compound of interest. Moreover, even if MSM produces a credible match, the resulting identification is only tentative as it is based on one dimension of supporting information only *(the spectral match itself)*. Part II of this series, *"Identification via Mass Spectral Matching"*, explains in great detail why it may not always be possible to come to an unequivocal identification for the targeted compound via an *MSM* identification strategy.

In either case, securing an identity when *MSM* fails – or elevating a tentative identification secured by *MSM* – an alternative identification strategy involves an expert interpretation of the mass spectrum's individual features *(mass values and their relative abundances)*. This approach of mass spectral interpretation is also often the basis for Mass Spectral Identifications in LC/MS as no universal commercial MS-libraries are available to support a mass spectral matching exercise for this technique. While it is not the intent of this document to provide a comprehensive and detailed discussion of all the fundamentals of mass spectral interpretation (*MSI*), essential principles and practices are discussed and illustrated.

In general, the *MSI* identification consists of three consecutive steps:

1. Determining which ion peak in the spectrum corresponds to the molecular weight of the molecule. In the case that the spectrum is acquired with an accurate mass high resolution instrument, the mass-to-charge ratio *(m/z)* of that ion can be used to generate a candidate molecular formula.

2. Establishing whether the compound of interest contains certain elements, such as chlorine or bromine, which have specific isotope patterns that translate into recognizable spectral features—namely specific relative abundances of monoisotopic masses.

3. Performing *de novo* structural elucidation. All peaks in a mass spectrum with an m/z value below that of the molecular ion are formed during the ionization *(or MS/MS fragmentation)* of the compound of interest and relate in one way or another to substructures or functional groups of that substance. An expert in fragmentation chemistry can relate this information to the molecular ion and potentially propose a

tentative structure via a process that is generally referred to as "*de novo* structural elucidation". Although algorithms have been developed to assist in certain aspects of such an elucidation, it should be emphasized that structural elucidation is always a subjective interpretation performed by an expert mass spectrometrist; therefore, any identity secured by structural elucidation is classified as being a tentative identification until additional collaborating data provides an upgrade of the identification level.

IDENTIFICATION OF THE MOLECULAR FORMULA

Identifying the elemental composition *(molecular formula)* of an unknown from its mass spectrum typically starts with determining which ion peak in the mass spectrum represents the m/z value of the intact, ionized molecule termed the molecular ion. In many cases, this determination is not as simple as just picking the highest m/z value in the spectrum. The ability and strategies to ascertain the *(pseudo)* molecular ion depend heavily on the type of ionization technique used.

GC/MS spectra are generally acquired with electron ionization *(EI)* which produces a radical molecular ion $M^{+\bullet}$ with highly variable intensities. The intensity of the molecular ion depends on its propensity to decompose into several smaller fragments which in turn are dictated by the stability of the ion under the applied ionization conditions. For instance, the molecular ion is usually very intense for compounds which are highly stable under certain ionization conditions, such as *(poly)*aromatics *(example shown in Figure 1A)*; while it is often not detected in spectra of largely unstable compounds such as aliphatic alcohols, highly branched compounds, and polyether glycols *(example shown in Figure 1B)*. Therefore, an independent assignment of the molecular ion for EI spectra, while not impossible, can be prone to the error of incorrectly picking an m/z value which is, in fact, associated with a fragment of the molecular ion.

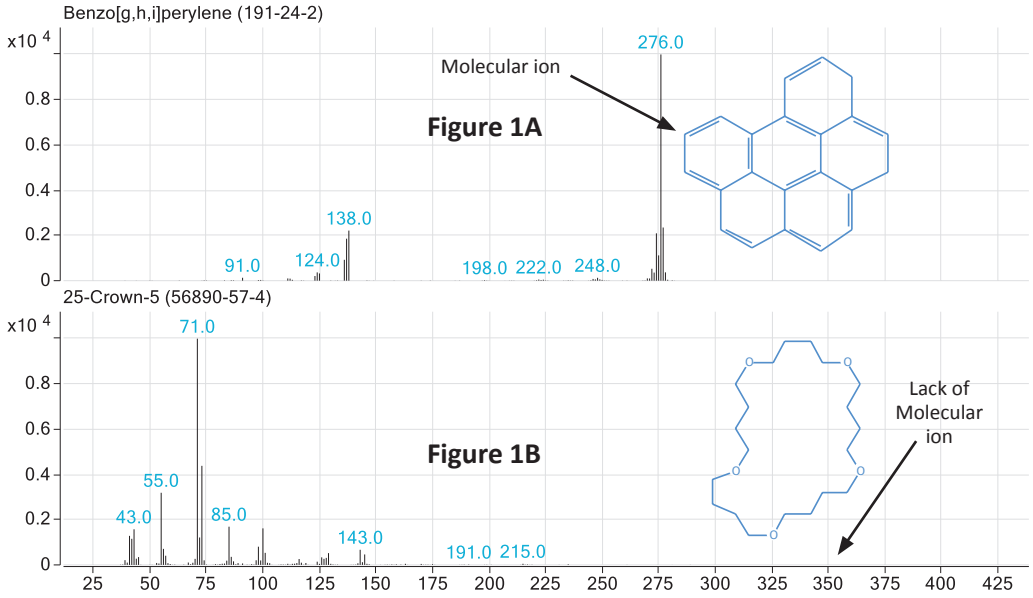

Figure 1: *Variability in intensity of the molecular ion in EI mass spectra demonstrated for a polyaromatic aromatic hydrocarbon, Benzo[g,h,i]perylene (Figure 1A), and a crown ether, 25-Crown-5 (Figure 1B). Benzo[g,h,i]perylene ($C_{22}H_{12}$) shows a clear molecular ion at m/z 276, whereas the molecular ion expected for 25-Crown-5 ($C_{20}H_{40}O_5$) at m/z 360 is not detected.*

More intense molecular ions are usually produced by "soft" ionization techniques such as atmospheric pressure chemical ionization *(APCI),* electrospray ionization *(ESI)* for LC/MS methods, and chemical ionization *(CI)* for GC/MS methods. Ionization of the molecule can result in protonated $[M+H]^+$ or deprotonated $[M-H]^-$ ions depending on the polarity of ionisation. In addition, adducts may be formed during ionization by reaction or clustering of the molecule with chemical entities present in the sample or mobile phase or due to reaction with a reagent gas. For instance, adducts with alkali or ammonium salts (*e.g. Na^+, K^+, NH_4^+ in positive mode, Cl^- in negative mode)* are frequently observed in APCI or ESI spectra. An example of this general phenomenon is given in Figure 2.

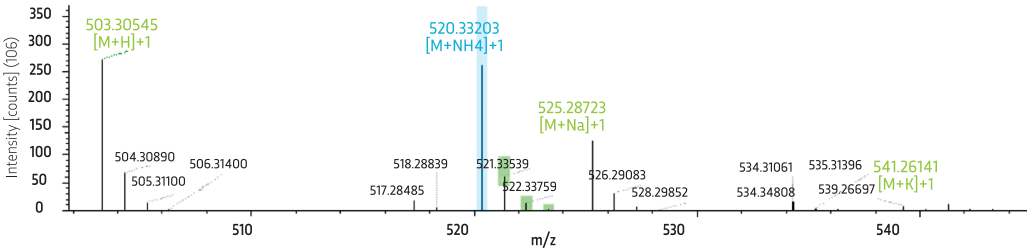

Figure 2. *Example of a positive mode ESI high resolution accurate mass spectrum of a compound illustrating formation of the pseudo molecular ion ([M+H]+) and concurrent adduct formation with alkali ([M+K]+1; [M+Na]+1) and ammonium ([M+NH4]+1) salts. The Ion at m/z 503,30545 will be used for further evaluation in Table 1.*

In the case of CI spectra *(e.g. in GC/MS)*, the protonated molecule often co-occurs with adducts between the molecule and the ionized reagent gas. An example of this is given in Figure 3. In addition to adducts, soft ionization may also be associated with in-source fragmentation depending on the ionization conditions, the stability of the *(pseudo)* molecular ion and, in case of CI, on the proton affinity of the molecule. In general, a thorough evaluation of adducts and in-source fragments is necessary to confirm the molecular ion.

Additionally, dimeric ions or even higher clusters can also be formed in the case of APCI or ESI.

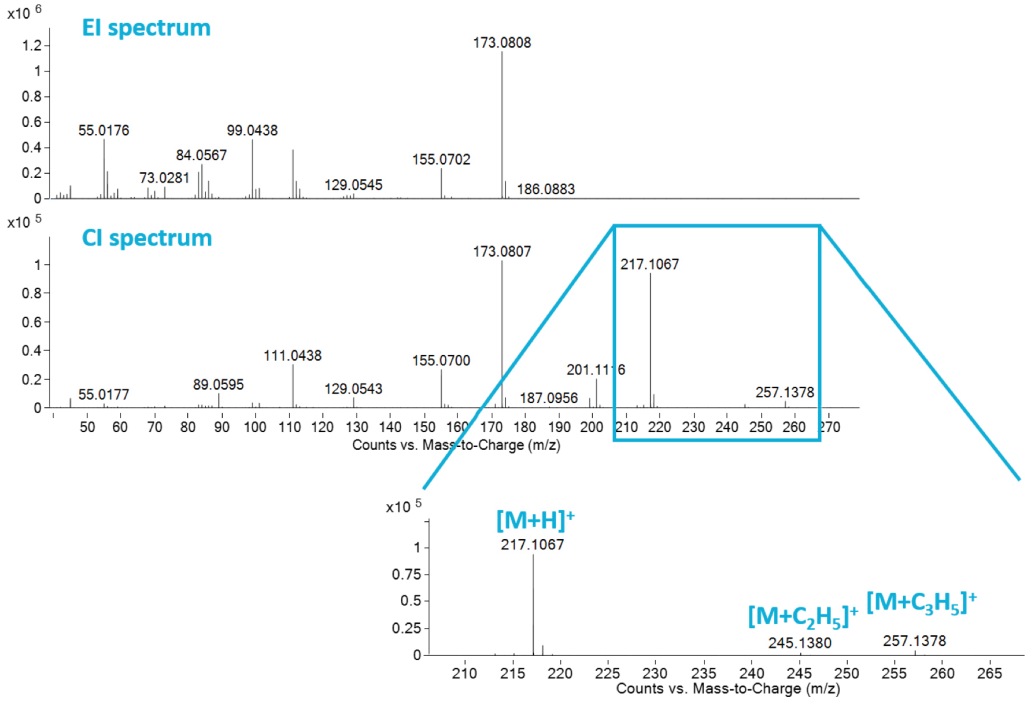

Figure 3: *Identification of the molecular ion of 1,4,7-Trioxacyclotridecane-8,13-dione ($C_{10}H_{16}O_5$) based on CI mass spectra in GC/MS. While the EI spectrum (top) shows no clear molecular ion, the CI spectrum (bottom) shows a clear protonated molecular ion $[M+H]^+$ at m/z 217.1067. CI data were acquired with methane as reagent gas which favors the formation of typical methane adducts. These were detected at m/z 245.1380 ($[M+C_2H_5]^+$) and m/z 257.1378 ($[M+C_3H_5]^+$) and thus reinforce the identification of the molecular ion.*

For both hard and soft ionization technologies, it should be emphasized that identifying the molecular ion in a mass spectrum is a subjective interpretation performed by a mass spectrometry expert; thus, there is a degree of uncertainty in the interpretation. In many cases, this uncertainty will be greater for electron impact ionization than it is for soft ionization techniques. Unfortunately, the degree of uncertainty cannot easily be expressed as a mathematical number such as the probability score used in mass spectral matching *(see Part II: "Identification via Mass Spectral Matching")*.

Although establishing the *(pseudo)* molecular ion with unit mass resolution is a significant step in compound identification, such information by itself is rarely adequate to secure even a tentative identification. However, if the molecular weight of the ion could be established with a high degree of resolution, the exact *(or accurate)* mass so secured could be used to generate a short list of candidate compounds whose molecular formulas have molecular weights equal to the determined accurate mass. An example of such a table where "candidate" elemental formulas are ranked, based upon the deviation of their calculated m/z compared to the measured m/z, can be found in Table 1. This exact mass information can be obtained with a high resolution - accurate mass spectrometer *(HRAMS)* such as time-of-flight, orbitrap, or ion cyclotron resonance mass spectrometers. The selectivity and mass accuracy of HRAMS instrumentation enables the distinction between candidate elemental composition formulas that would be undistinguishable on unit mass instrumentation obtained from quadrupole or ion-trap-based mass spectrometers. On such instrumentation, ions that only slightly differ in m/z value would be detected as isobaric signals. For example, diethyl fumarate and 2-fluorobiphenyl have the same unit mass of 172 Da and thus would be indistinguishable on that basis alone. However, their accurate masses *(172.0730 Da and 172.0683 Da, resp.)* are sufficiently different that they would be readily distinguished on the basis of the elemental compositions obtained using HRAMS since such mass measurements enable the determination of the ions elemental composition by considering the sum of the exact masses of various nuclides *($C_8H_{12}O_4$ and $C_{12}H_9F$, respectively).*

The generation of molecular formulas from accurate mass information is usually assisted by software algorithms using user-defined search constraints. Search criteria include the species and quantity of allowed elements, allowed mass accuracy *(depends on the resolution of the mass spectrometer)*, the charge state *(e.g. singly or multiply charged),* and the allowed electron state, which refers to the number of electrons *(even or odd)* and depends on the ionization technique. Soft ionization techniques normally produce ions with an even electron state. EI spectra, on the other hand, generate a radical molecular ion with an odd number of electrons, while fragments of the molecular ion can either have an odd or even number of electrons. For the ESI-HRAMS example in Figure 2, several even electron elemental formulas can be predicted for the pseudo molecular ion detected as $[M+H]^+$ at m/z 503.30545, see Table 1 below.

	Elemental Formula	Theoretical m/z	RDB	m/z Deviation (ppm)
1	C24 H39 O4 N8	503.30888	9.5	-6.811
2	C23 H43 O8 N4	503.30754	4.5	-4.154
3	C22 H47 O12	503.30620	-0.5	-1.497
4	C19 H39 O6 N10	503.30486	5.5	1.181
5	C18 H43 O10 N6	503.30352	0.5	3.839
6	C30 H39 O3 N4	503.30167	13.5	7.515

Table 1. *Predicted elemental formulas and corresponding number of ring plus double bonds (RDB) values for an even electron ion with m/z 503.30545 considering C, H, O and N as allowed elements with a m/z deviation tolerance of 10 ppm.*

In addition to establishing molecular formulas, accurate mass information can even give structural information based on the number of *"ring and double bond equivalents"*-rule that is a conventional measure of the degree of the unsaturation of an organic molecule corresponding with the lowest formal valence state of the elements present in its elemental formula.

Depending on the mass resolution and mass accuracy of an HRAMS measurement and the structure of the compound of interest, it may or may not be possible to choose among the multiple candidate elemental formulas that are a reasonable match to the accurate mass established as the m/z peak of the *(pseudo)* molecular ion. In that case, the correct molecular formula can be established by evaluation of the isotopic data.

INTERPRETATION OF ISOTOPIC DATA

Most elements appear naturally as a mixture of isotopes of which the stable isotopes are of prime importance for identification purposes. For example, natural carbon is a mixture of 98.9 % of isotope ^{12}C and 1.1 % of isotope ^{13}C. The natural isotopic composition of a molecule is reflected in the mass spectrum by the presence of isotopic clusters. Such a cluster is composed of distinct monoisotopic masses with relative abundances that reflect their distinct isotopic compositions. By consensus, the first peak in the cluster of peaks corresponding to the most abundant isotopes of a given ion is designated as X. The isotopic regions in a mass spectrum that corresponds to 1 or 2 *(or more)* mass units further away from X are designated as X+1, X+2, etc. regions. The common elements such as C, H, N, and O – which have a diagnostic isotopic pattern with relatively low abundance of their first isotope at X+1 – do not show very intense and obvious isotopic compositions when measured as unit mass. On the contrary, certain elements such as Cl, Br, S, K and Si have very characteristic and intense isotopic distributions up to the X+2 region. For instance, bromine isotopes have a natural composition of 50.7 % ^{79}Br and 49.3 % ^{81}Br. Therefore, a molecular formula of $C_{13}H_{23}Br$ has an average molecular weight of 259.231 which in the mass spectrum will be observed as distinct peaks at 258.098 Da and 260.096 Da with almost equal intensities as shown in Figure 4.

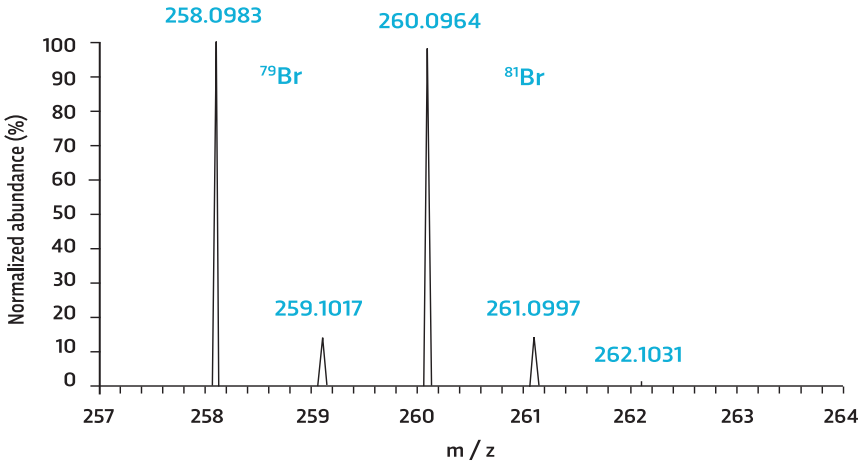

Figure 4. *Example of a diagnostic bromine isotope pattern for elemental formula $C_{13}H_{23}Br$ rubber oligomer. The almost equal size of the two chromatographic peaks represent the almost equal natural abundances of these isotopes (50.7 % ^{79}Br and 49.3 % ^{81}Br).*

These diagnostic isotopic clusters are readily recognizable by a mass spectrometry expert and can be used to reveal the presence of specific elements. In addition, algorithms have been developed to predict the presence of certain elements such as chlorine and bromine *(e.g. NIST/EPA/NIH MS Search software)*. Accurate mass data are not required to infer the presence of these elements from the above isotope patterns, although it would certainly reinforce the isotopic evidence for the presence of certain elements in other cases.

Take for example a *(pseudo)* molecular ion that is detected at m/z 177.0944 ± 0.0004 using a HRAMS measurement. Considering the mass accuracy of this HRAMS measurement, three candidate formulas are possible: $C_7H_{17}O_3Si$ *(m/z 177.0942)*, $C_8H_{17}O_2S$ *(m/z 177.0944)* and $C_6H_{11}N_4F_2$ *(m/z 177.0946)*. In this case the correct formula can only be identified by interpretation of the X+1 region's related isotopic pattern, i.e. around m/z 178.09. The theoretical isotopic clusters are shown in Figure 5A for $C_6H_{11}N_4F_2$, Figure 5B for $C_8H_{17}O_2S$ and Figure 5C for $C_7H_{17}O_3Si$. These clusters clearly illustrate that the expected differences between these isotopic patterns in the X+1 region can be used to select the correct formula by comparison with the X+1 region in the experimental spectrum for the compound of interest *(if the measurement's mass resolution and mass accuracy is sufficient).*

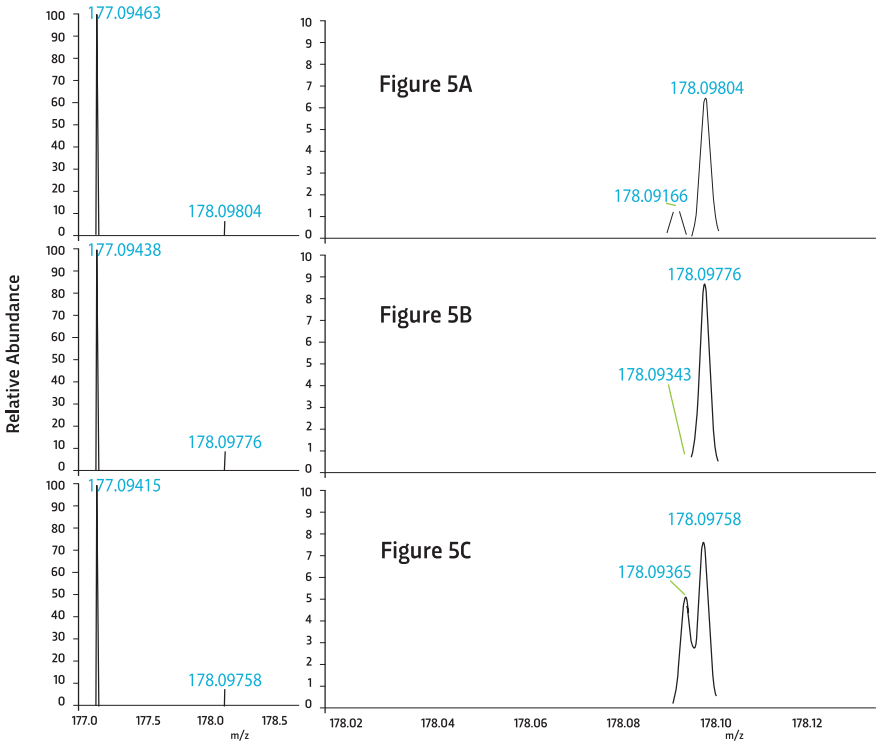

Figure 5. *Theoretical high-resolution accurate mass spectra for ions with an elemental formula of $C_7H_{17}O_3Si$ (Figure 5A), $C_8H_{17}O_2S$ (Figure 5B) and $C_6H_{11}N_4F_2$ (Figure 5C) for m/z range 177 – 178.5 and Right: the corresponding zoomed X+1 region around m/z 178.09 at 70000 resolving power.*

INTERPRETATION OF MASS FRAGMENTS: *DE NOVO* STRUCTURAL ELUCIDATION

The ion peaks present in a mass spectrum can be interpreted to establish the presence of functional groups or substructures in the compound of interest, to place the compound of interest into certain structure-based classes *(e.g. alcohols or phthalates),* or even to propose a tentative molecular structure. This is because the ions, formed during ionization, represent either the ionized molecule or ionized fragments thereof. Fragmentation of a molecule principally occurs in a predictable and reproducible way within the boundaries of the applied instrumental parameters. The general mechanisms for such fragmentation reactions have been extensively described in authoritative reference works on mass spectral interpretation [1, 2, 3, 6]. For example, cleavages resulting in the loss of neutral molecules *(water, carbon monoxide, methanol, etc.)* are usually produced by structural rearrangements or proton shifts of the ionized molecule. An example of such a cleavage can be found in Figure 6.

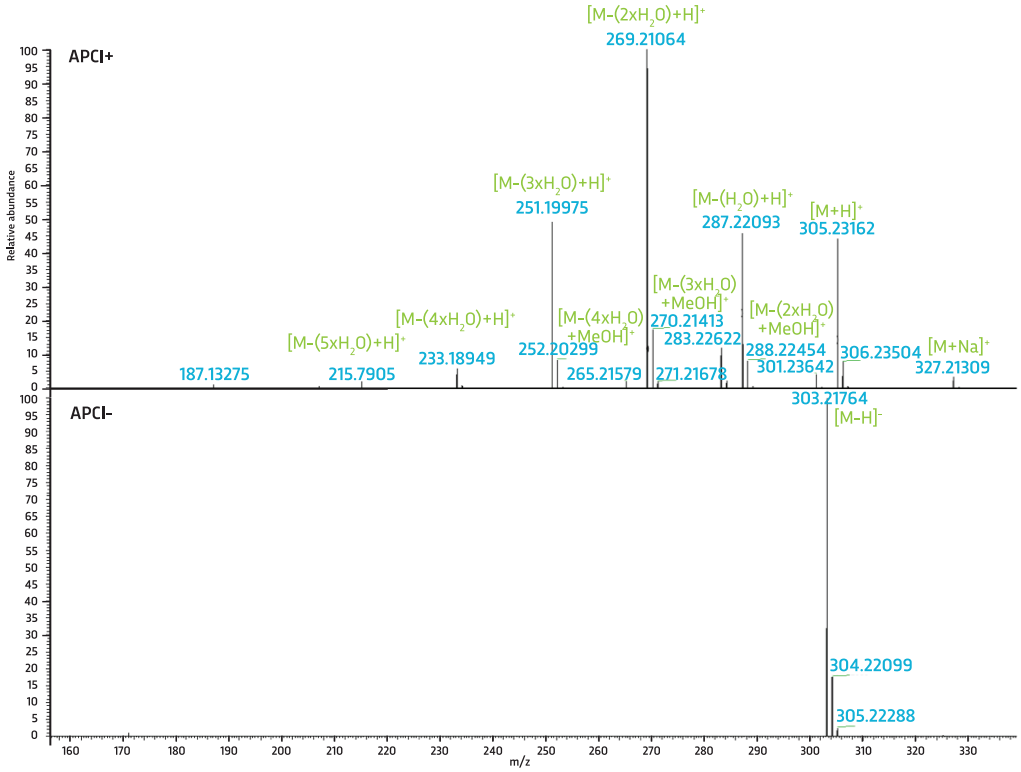

Figure 6. *Example of an annotated positive(top) and negative mode APCI high resolution accurate mass spectrum of a compound (aleuritic acid) illustrating formation of the pseudo molecular ion ([M+H]+, [M-H]-) concurrent adduct formation and loss of neutral fragments by in-source fragmentation.*

Another type of cleavage involves the loss of a radical fragment *(e.g. methyl radical)*, which is almost exclusively observed in EI spectra. A cleavage generates two types of informative spectral values namely, the mass value of the formed fragment and mass differences of that fragment with a heavier fragment or the molecular ion— commonly referred to as "losses".

An experienced mass spectrometrist who has an in-depth knowledge of fragmentation rules can relate observed mass values and losses to specific fragment structures which are linked together to establish a logical fragmentation pathway. Moreover, an extensive chemistry background is imperative to assess whether a proposed structure is viable, that it is thermodynamically stable, and whether it is likely to be detected with the applied technique.

The goal of structural elucidation is to elucidate as many fragments as possible and to link the fragments together via a rational pathway; so doing limits the number of possible structures to the smallest number of candidates. In general, the more fragments that can be fit into a defendable fragmentation pattern for a proposed structure, the greater the likelihood that the identity established by elucidation is, in fact, the correct identity.

In the initial stages of elucidation, numerous structure candidates can often be proposed which fit the generated molecular formula or observed fragments to varying degrees. As a general rule, the relative percentage of peaks that can be rationalized by a fragmentation pathway for a given structure is directly related to the likelihood that the spectrum indeed corresponds to that structure; that is, the higher the percentage of rationalized peaks, the greater the likelihood that the elucidated identity is the correct identity. A complicating factor in structural proposal, however, is that not all structures have unique mass spectra. This is often the case for compounds with very similar structures. For instance, the degree and position of branching of hydrocarbon chains or the exact stereochemistry of a molecule often cannot be inferred from a spectrum. Therefore, the confidence level of identifications which are solely based on structural elucidation is limited to a TENTATIVE identification at best. This is the case even for compelling elucidations, as the identification is still based only on one dimension of information. A higher level of confidence can be achieved by gathering additional data such as retention time, MS/MS spectra, or spectra recorded with a different type of ionization. In addition, other corroborating data, such as the result of an identification found in another orthogonal and complementary technique *(such as GC/MS identification results for LC/MS compound identifications)*, disclosed compositional data of the material of construction, or other analytical techniques that can assist in the confirmation of the elucidated structure *(e.g. NMR on the isolated compound),* can assist in upgrading the elucidated structure from a tentative to a confident or confirmed identity *(see also Part IV: "Additional Evidences supporting a Higher Level of Identification"*). It goes without saying that the highest level of identification is obtained by confirming the mass spectrum *(and associated retention time)* of the tentatively identified compound with its authentic standard, providing sufficient corroborating information, or both so that the chances of an incorrect identification are small.

CASE STUDIES

The ability and strategy to propose an initial tentative molecular structure depend largely on the ability to identify the molecular ion and molecular formula and the availability of reference mass spectra that are similar to the spectrum of the unknown. The following three cases demonstrate these strategies:

CASE 1: MOLECULAR ION NOT IDENTIFIED

As mentioned previously, the likelihood of detecting and identifying the molecular ion depends on the ionization technology *(EI, CI, APCI, ESI, etc.)* and on the stability of the ionized molecule. If the molecular ion cannot be identified, potentially all peaks in the spectrum are fragments of a larger molecular structure. In that case, any proposal of a molecular structure would be highly speculative. At best, indications for the presence of functional groups, substructures, or general compound classes could be inferred based on the similarity of spectral features with available reference mass spectra. The underlying principle is that spectra from molecules with very similar structures also have similar spectral features. This is particularly relevant for spectra of unknowns which are not present in a library of reference spectra. The degree of similarity is not limited to the observation of equal mass values or relative abundances in either measured or the reference spectrum, it may also include equal losses.

Because of the inability to propose a structure, identifications secured in this manner are classified as PARTIAL identifications. Some examples of these partial identities include:

- EI spectra of phthalate esters contain an intense m/z 149 ion which is often the only major peak. The molecular ion is often not detected.
- EI spectra of aliphatic hydrocarbons are characterized by a typical pattern of m/z 43, 57, 71, 85 etc.
- The presence of ions m/z 77 and 91 in an EI spectra is diagnostic for the presence of phenyl and benzyl substructures, respectively.
- CI Spectra that contain a mass difference of 18 Da indicates the loss of water which is typically observed in alcohols or acids but not in ketones.

Another example of a partial identification for a siloxane compound is shown in Figure 7. Experienced mass spectrometrists will recognize such spectral similarities more readily than will less-experienced analysts. Alternatively, software tools have been developed to assist with such substructure identifications. For example, the substructure analysis tool in the NIST/EPA/NIH MS Search software analyses the presence of substructure signatures in the hit list of a particular unknown spectrum and the match of the different hits to the unknown [4]. This analysis is then translated to a list of probabilities of substructures being present and absent in the unknown spectrum.

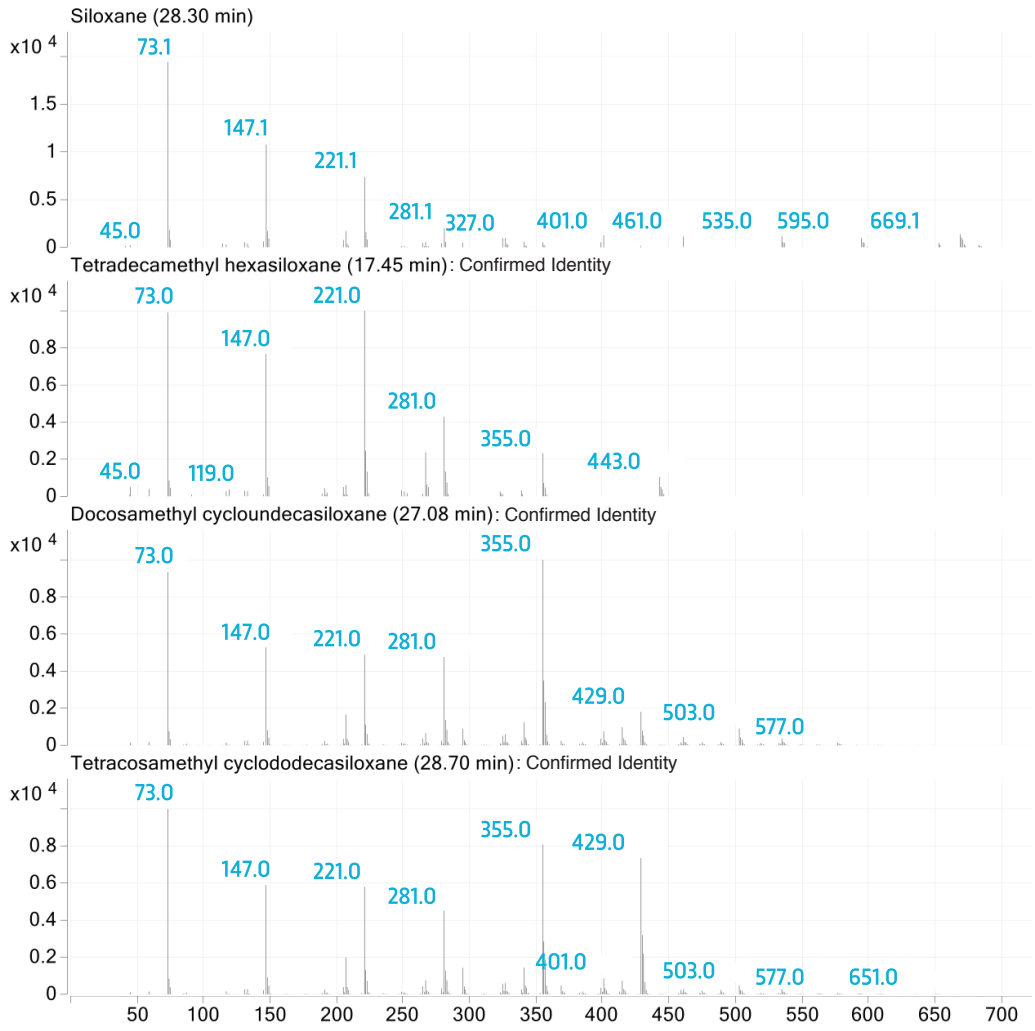

Figure 7. *Example of a partial identification in which the mass spectrum of the unknown (top spectrum) contains typical fragments with m/z values 73, 147, 221 and 281. The mass spectra of siloxanes, confirmed with authentic standards, are shown for comparison and have the same characteristic pattern. This justifies a partial identification, particularly because neither the spectrum nor the retention time yields a perfect match with any of the confirmed siloxanes.*

CASE 2: MOLECULAR ION IS IDENTIFIED (UNIT MASS)

All strategies described previously can also be used when the molecular ion can be identified but the molecular formula cannot be established *(e.g. low-resolution GC/MS)*. In this case, however, knowledge of the molecular ion adds the possibility of relating all evident substructures to a certain molecular weight. In addition, a good but not perfect mass spectral match with a spectrum from an external reference library could be a reference to assist in the structural elucidation. Once the fragmentation pathways of the matching compound can be elucidated, rationalizing mutual spectral differences between unknown and matching compounds may lead to the proposal of a tentative structure. The process of rationalizing all spectral peaks for a structure candidate can be facilitated by using software packages specifically designed for *in silico* fragmentation prediction such as MS Interpreter *(NIST/EPA/NIH), MS Fragmenter (ACD Labs) or Mass Frontier (Thermo Scientific / HighChem LLC).* An example of rationalizing different mass spectral fragments through the use of a software package can be found in Figure 8

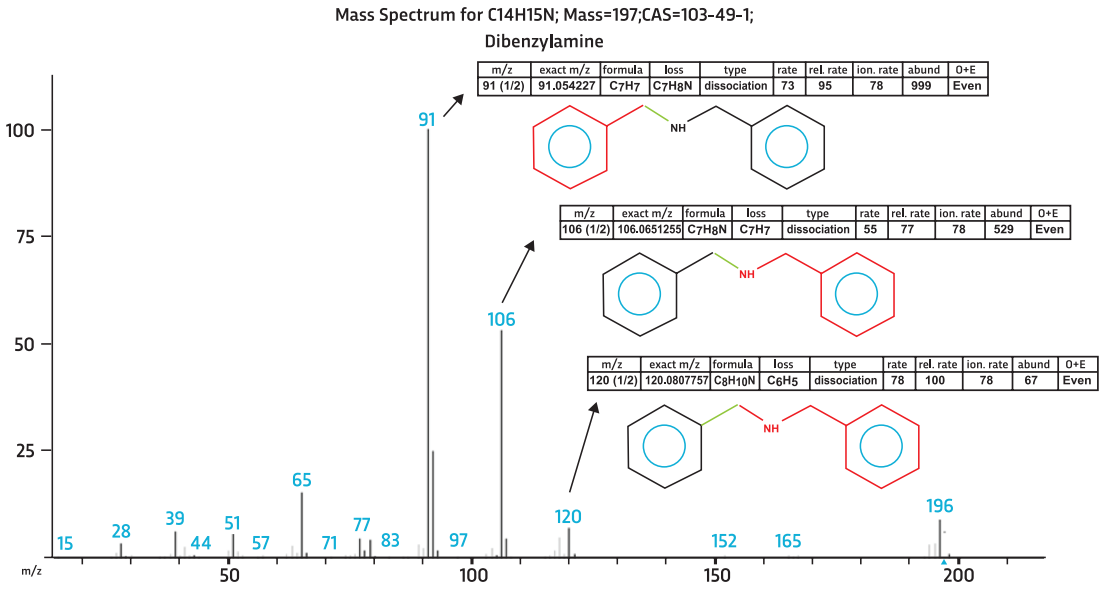

Figure 8. *Explanation of fragmentation pathways observed in the EI mass spectrum of dibenzylamine using MS interpreter (version 3.4b, NIST/EPA/NIH). For instance, the base peak ion m/z 91 as well as fragment ions m/z 106 and m/z 120 can be explained by bond cleavages by dissociation at different positions of the molecule (fragment structures are displayed in red).*

CASE 3: MOLECULAR FORMULA IS IDENTIFIED (ACCURATE MASS)

In cases where the molecular formula can be determined from the mass spectrum, it is often possible to draw numerous structures which comply with the elemental composition of the molecule. Therefore, a molecular formula on its own can only correspond to a partial identification level. Databases such as PubChem, SciFinder, or Chemspider could be used to search known structures. However, it should be noted that the actual structure might not be present in these databases as the number of known chemical structures is only a fraction of the total chemical space of organic compounds. [5] To reduce the number of structure candidates, mass fragments should be interpreted within the boundary of the identified molecular formula. In this case, the availability of accurate mass data increases the confidence of relating fragment ions and hence their elemental composition to the molecular structure. In addition, mass differences can more easily be linked to small functional groups. For example, an integer loss of 28 Da can either reflect the loss of ethylene *(C_2H_4, 28.0313 Da)* or carbon monoxide *(CO, 27.9949 Da).*

Other means for confirming the proposed elucidated structure include, but are not limited to:

- the use of available corroborating data such as supplier information on the composition of the material
- identification results of other chromatographic techniques used in the characterization process
- other techniques used to elucidate chemical structures *(e.g. NMR).*

The approach of using additional evidences to support a higher level of identification is described in Part IV of this series of white papers on Good Identification Practices.

CONCLUSION

The use of mass spectral detectors in the screening process to identify all organic Extractables and Leachables that are present in a material, component or device – or compounds that may have leached out and lead to patient exposure – has been widely accepted and implemented.

It is generally accepted that mass spectral matching is a fairly reliable means of identifying organic extractables and leachables via GC/MS. However, there are many circumstances where mass spectral matching will not lead to the desired outcome of an unequivocal identification *(see Part II: "Identification via Mass Spectral Matching"*). In such cases, more information about the compound's identity can be obtained via mass spectral interpretation. It should be noted that for mass spectra generated via LC/MS, mass spectral interpretation is often the only way to increase the level of identification for a detected compound, as there are no universal mass spectral libraries available that are optimized for LC/MS.

A key success factor in mass spectral interpretation is establishing the molecular ion of the detected compound. If the molecular ion is established with a High-Resolution Accurate Mass Instrument, then possible elemental compositions of the compound can be secured. In that case, additional information such as interpretation of isotopic data and interpretation of mass fragments can lead to elucidation of the compound's structure. Additionally, publicly available

databases *(such as PubChem, SciFinder or ChemSpider)* can be consulted to look for actual structures that could potentially fit the generated mass spectrum. In addition, existing software packages - using in-silico fragmentation - can assist in rationalizing all mass spectral peaks for a structure candidate and support or reject the validity of the suggested structure. When the fragmentation supports the suggested structure *(either via manual expert interpretation or via software supported interpretation)*, you can consider a "*De Novo*" structural elucidation. In that case, the identification by structure elucidation may lead to a tentative identification. A further increase in identification level from tentative to confident or confirmed can be accomplished through the use of corroborating data *(see also Part IV: "Additional Evidences supporting a Higher Level of Identification")* or through the analysis of an authentic standard for the suggested compound.

When the elemental formula cannot be derived from the corresponding mass spectrum, then any proposal of a molecular structure would be highly speculative, and the highest level of identification that can be obtained from this information is a "partial" identification.

If "unit mass" mass spectrometers are used to collect the identifying information, a full structural identification of the compound will rarely be the outcome unless spectra of structural analogs of the compound of interest are present in a spectral library and the compound of interest is subject to similar fragmentation pathways. Generally, higher resolution, so-called accurate mass data will be required to complete the structure elucidation and thereby generate a tentative identity.

It should be emphasized that postulating a chemical structure solely based upon mass spectral information and interpretation is not an easy task. The mass spectrometrist should be aware of the importance and consequences of postulating a defined chemical structure, as this information will be used to link the chemical compounds to its toxicological information and will be the basis for a subsequent toxicological evaluation of the compound. Cases where the wrong identity for the compound is postulated will inevitably bias the overall safety evaluation of the material, device, or container/closure component or system.

MOVING FORWARD

The use of mass spectral matching with commercially available mass spectral databases is often an effective means of securing an acceptable, albeit tentative, identity for an organic extractable or leachable, as outlined in Part I of this series. Part II of this series considered both the merits of such an approach and some points of attention and potential pitfalls of such an identification strategy. In Part III, an identification strategy based upon mass spectral interpretation was outlined. Although this approach is often an effective means of securing acceptable tentative identities, the process of mass spectral interpretation is challenging and securing even a tentative identity based upon the isolated information that is made available through the compound's mass spectrum is not assured.

In the last Part *(Part IV)* of this series, we will discuss the additional and supporting data that can be used to corroborate tentative identifications obtained through either mass spectral matching or interpretation. We will establish how such corroborating information can be used to increase the likelihood that a tentative identity is in fact the correct identity, resulting in either confident or confirmed identifications.

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